UVA Radiation Impairs Phenotypic and Functional Maturation of Human Dermal Dendritic Cells

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There is now strong evidence that the ultraviolet A (UVA) part of the solar spectrum contributes to the development of skin cancers. Its effect on the skin immune system, however, has not been fully investigated. Here, we analyzed the effects of UVA radiation on dermal dendritic cells (DDC), which, in addition, provided further characterization of these cells. Dermal sheets were obtained from normal human skin and irradiated, or not, with UVA at 2 or 12 J per cm^2. After a 2 d incubation, the phenotype of emigrant cells was analyzed by double immunostaining and flow cytometry. Results showed that migratory DDC were best characterized by CD1c expression and that only few cells co-expressed the Langerhans cell marker Langerin. Whereas the DC extracted from the dermis displayed an immature phenotype, emigrant DDC showed increased expression of HLA-DR and acquired co-stimulation and maturation markers. We showed here that UVA significantly decreased the number of viable emigrant DDC, a process related to increased apoptosis. Furthermore, UVA irradiation impaired the phenotypic and functional maturation of migrating DDC into potent antigen-presenting cells, in a concentration-dependent manner. The results provide further evidence that UVA are immunosuppressive and suggest an additional mechanism by which solar radiation impairs immune response.

Key words: dendritic cells/dermis/immune suppression/UVA
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Exposure to ultraviolet (UV) radiation is the major cause of most skin cancers. Historically, most studies have concentrated on UVB (290–320 nm), which constitute the most energetic part of the solar spectrum at the earth's surface. Based on DNA absorption spectrum, UVB directly induced mutations and chromosomal damage and was therefore considered more carcinogenic than UVA (320–400 nm) (De Gruijl *et al*, 1993). Furthermore, UVB is highly immunosuppressive, a process that plays a key role in tumor progression. UVB-induced immunosuppressive effect has been well characterized and related to multiple mechanisms including DNA damage, cytokine production, and direct alteration of Langerhans cell (LC) antigen-presenting function (Duthie *et al*, 1999).

UVB radiation, however, only comprises about 5% of the solar UV that reaches the biosphere, whereas UVA are 20 times more abundant. Furthermore, UVA penetrates deeper into the skin and, upon exposure to sunlight, 100 times more UVA than UVB photons reach the dermis. UVA radiation has been shown to cause DNA damage and mutations, mostly through the generation of oxygen/nitrogen species, which then interact with various molecular components (Halliday *et al*, 1998). Recently, there has been compelling evidence that UVA contributes to UV-induced immune-suppression. Indeed, UVA exposure of human skin results in decreased induction of contact sensitisation and induced tolerance (LeVee *et al*, 1997; Moyal and Fourtanier, 2001). UVA is the critical wavelength for suppressing established immune response to Candida albicans in mice (Nghiem *et al*, 2001). Furthermore, sunscreen studies indirectly confirmed that UVA are immunosuppressive by showing that products with high UVA absorption afforded a higher degree of immune protection than the products with low UVA protection, despite equal filtration of UVB (Damian *et al*, 1997; Moyal and Fourtanier, 2001; Baron *et al*, 2003; Poon *et al*, 2003). Despite the demonstration of UVA overall immunosuppressive effect, the impact of the radiation on the skin immune system has received relatively little research attention. The few studies only concern epidermal LC, whose allostimulatory function was altered by UVA (Clement-Lacroix *et al*, 1996).

Due to their accessibility, LC are the best-characterized DC. The cells exhibit some characteristic features such as expression of CD1a and Langerin, a major constituent of Birbeck granules (Valladeau *et al*, 2000). Moreover, numerous studies have established the critical role of LC in initiating skin immune responses, due to their ability to take up antigens that cross the epidermal barrier, migrate to regional lymph nodes, and present pre-processed antigens to naïve T cells (Steinman, 1991). By contrast, DC populating the dermis are still poorly defined. Early studies have demonstrated their potent role in skin sensitization to allergens (Streilein, 1989), or antigen presentation (Sepulveda-Merrill *et al*, 1994; Nestle *et al*, 1998). Some recent papers suggest, however, that dermal DC (DDC) may differ from LC regarding antigen presentation (Pena-Cruz *et al*, 2003; Ritter *et al*,

Abbreviations: DDC, dermal dendritic cells; LC, Langerhans cells; UV, ultraviolet

2004) or Th1 *versus* Th2 orientation of the T cell response (Strid *et al*, 2004). Since UVA enters the dermis, DDC might be potent targets of the radiations. In this study, we sought to analyze the effects of UVA radiation on human DDC, which, besides, will provide further characterization of these cells.

Results

Characterization of emigrant dermal cells We first characterized, by flow cytometry, the cells that had spontaneously migrated from the dermis after a 48 h culture in serum-free medium. Viability of the cells always exceeded 90%, as assessed by trypan blue exclusion. According to the donors, the mean number of migratory cells per gram of dermis was $5 \times 10^5 \pm 7 \times 10^4$ ($n = 6$). In eight experiments, the relative proportion was 21% ± 11% $CD3^+$ T cells, 2.4% ± 1.1% $CD19^+$ B lymphocytes, and 8.0% ± 5.2% $CD14^+$ monocytes/macrophages. $CD16^+$ cells, i.e., neutrophil granulocytes and macrophages ($CD36^+$ cells) were present in a negligible proportion. Importantly, Langerin-positive LC never exceeded 4% of the total migrating cells. $CD45^-$ cells, most probably keratinocytes and fibroblasts, represented between 15% and 35% of the total cell suspension. Finally, the percentage of DC, as identified by CD1c expression at their surface, averaged 52% ± 10%. Under a phase-contrast microscope, the cells could be easily identified by their dendritic processes and quite resembled cultured LC (not shown).

Using flow cytometry (FACS) analysis, the dendritic leukocytes could be easily distinguished from most other cell types by forward scatter (FSC) and side scatter (SSC) profiles. As depicted in Fig 1*A*, small cells, corresponding to B and T cells, could be easily distinguished from the large cells corresponding to DC. $CD45^-$ non-leukocyte population was scattered in the FSC/SSC plot, but quite excluded from the dendritic population. By contrast, $CD14^+$ cells were included in the DC cloud.

Double staining showed that a large majority of $CD45^+$ and HLA-DR^+ cells were DC, as they co-expressed CD1c or CD1a, the other HLA-DR^+ populations being identified as T cells, B cells, or monocytes (Fig 1*B*). In fact, in five experiments, the total amount of HLA-DR^+ cells (71.3% ± 5.1%) was equivalent to the total of HLA-DR^+/$CD1c^+$ DC (57.6% ± 5.3%), HLA-DR^+/$CD3^+$ T cells (4.1% ± 2.5%), HLA-DR^+/$CD14^+$ monocytes (9.1% ± 1.9%), CD16 + neutrophils (1.1% ± 0.9%) and HLA/DR^+/$CD20^+$ B cells (1.0% ± 0.8%), therefore demonstrating that all dermal DC are in the $CD1c^+$ population. Virtually, all $CD1c^+$ cells were $CD11b^+$ and $CD11c^+$. None of $CD1c^+$ cells expressed the CD3 T cells marker. All the $CD1a^+$ cells were $CD1c^+$, whereas a minor population of $CD1c^+$ (5%–10%) cells did not express CD1a (Fig 1*B*). Very few cells co-expressed CD1c and CD14 antigens, or CD1c and Langerin, whereas most $CD1c^+$ cells expressed the factor XIIIa (Fig 1*B*). Interestingly, CD36 expression was found on a small percentage of dermal $CD1c^+$ DC as well as $CD1a^+$ DC (not shown). It should be noted that DC-SIGN expression was undetectable on the migratory dermal cells (not shown).

CD1c is known to be present at the surface of a small subset of B cells. In our experiments, however, neither $CD20^+$ (Fig 1*B*) nor $CD19^+$ B cells (not shown) were $CD1c^+$, suggesting that the B subset is not present in the dermis.

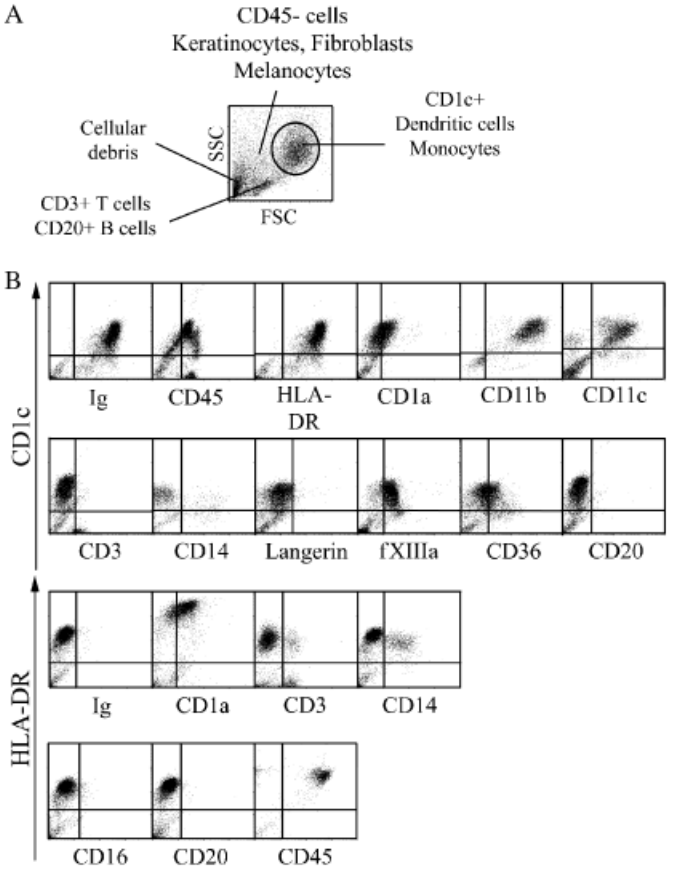

Figure 1
Phenotypic profile of dermal migrating cells. After 48 h incubation of human dermis in serum-free medium, migrating cells were recovered and processed for flow cytometry. (*A*) Forward and side scatter identification of the different cell subsets, including dermal dendritic cells (within the gate). (*B*) Double staining of dermal cells with either anti-CD1c or anti-HLA-DR and the indicated monoclonal antibodies. The total dermal cell suspension was analyzed without any gating. The quadrant gates were set up according to the negative control Ig. The results are representative of at least seven experiments carried out with different donors.

UVA radiation induces DDC apoptosis We investigated whether UVA irradiation would modify the total number or proportion of emigrant viable dermal cells. In a series of six experiments, the number of viable emigrant cells per g of dermis was not altered after irradiation at 2 J per cm^2. By contrast, it was significantly decreased after irradiation at 12 J per cm^2 (263.8×10^3 *vs* 499.8×10^3 cells per g of dermis, $p < 0.001$).

The relative proportion of $CD3^+$ T cells was increased (18.7% ± 9.8%, 30.4% ± 6.2%, and 27.1% ± 6%), whereas that of $CD1c^+$ was concomitantly decreased (56.6% ± 9.3%, 43.4% ± 9.3%, and 33.9% ± 9.2%) after irradiation at 0, 2, and 12 J per cm^2, respectively (mean from five experiments).

Given the concentration-dependent decrease in the total number of emigrant cells and in the proportion of DDC, we hypothetized that this effect might be due to an induction of cell apoptosis. To verify this possibility, cells were stained

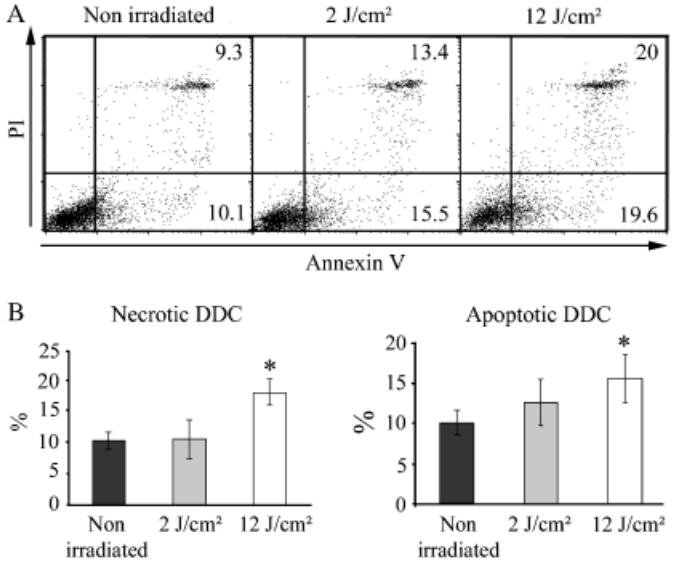

Figure 2
Ultraviolet A (UVA) irradiation promotes the apoptotic death of human dermal dendrtic cell (DDC). Human dermis was irradiated, or not, with UVA at 2 or 12 J per cm^2 and incubated for 48 h in serum-free medium. Migrating cells were then recovered and analyzed for apoptosis using annexin V and propidium iodide staining. (*A*) DDC were analyzed by flow cytometry, after gating on FSC/SSC parameters according to Fig. 1*A*. Apoptotic cells are located in the lower right quadrant and necrotic cells in the upper right quadrant. Numbers represent the percentage of cells in each quadrant. (*B*) Results from four independent experiments showing the mean percentage ± SD of apoptotic and necrotic cells, as defined in *A*. *Results statistically different ($p < 0.005$) from the non-irradiated control.

using Annexin V—fluorescein isothiocyanate (FITC) monoclonal antibody (mAb) and propidium iodide (PI). Cell analysis was then carried out on either the total migrating cell population or DDC, by gating the cells according to FSC/SSC parameters, as described in Fig 1*A*. In a series of four experiments, we observed that the percentage of dead and apoptotic cells in the total dermal cell population tended to increase upon UVA irradiation, but the effect was not significant, as assessed by Student's *t* test (data not shown). When we focused on DDC, however, we observed a significant increase ($p < 0.05$), in the percentage of both apoptotic and necrotic cells (Fig 2*A* and *B*) after irradiation at 12 J per cm^2. The data suggest that the higher dose of UVA increases DDC death, in a significant way.

UVA irradiation impairs the maturation of human migrating dermal DC In order to assess the effects of UVA radiation on the activation/maturation markers on migrating DDC, we first analyzed the phenotypic status of freshly isolated DDC. After enzymatic digestion of human dermis, resident DDC represented about 10% of the dermal cell population, as identified by CD1 expression (9.2% ± 1% $CD1a^+$ and 11.5% ± 1.1% $CD1c^+$ cells, mean from three experiments). Double staining (Fig 3*A*) showed that all $CD1c^+$ or $CD1a^+$ cells (not shown) co-expressed CD45 and HLA-DR, whereas none of them were $CD3^+$, $CD14^+$, or $CD20^+$. CD36 was expressed on about half the CD1c population. Whereas almost all $CD1c^+$ were factor $XIII^+$ (not shown), very few cells (about 6%) co-expressed Langerin. In agreement with a recent paper (Turville *et al*, 2002), we found that only a minor DDC subset (about 7%) were DC-$SIGN^+$. Interestingly, we consistently noticed a DC $SIGN^+/CD1c^-$ population. Using triple staining, this population was identified as $CD45^-$ and most probably represented endothelial cells (not shown).

As shown in Fig 3*B*, most of the freshly isolated $CD1c^+$ DC are immature since they did not express CD40, CD54, CD80, CD83, CD86, CCR7, and DC-LAMP Ag. By contrast, upon 48 h incubation, emigrant DDC acquired a mature phenotype, as evidenced by higher expression of HLA-DR, as well as acquisition of most above-mentioned Ag (Fig 3*B*). Although CD1c expression remained constant, CD1a expression was downregulated on migratory, as compared with resident DDC (Fig 3*B*). When the protease digestion cocktail was added to mature migrating dendritic cells, the treatment did not markedly alter the DDC phenotype (not shown), thus demonstrating that the immature state of freshly isolated DDC was not related to protease treatment.

We next analyzed the effects of UVA on the phenotype of emigrant DDC using double staining and gating on $CD1c^+$ cells. Table I gives the mean results from at least four independent assays. As compared with sham-irradiated cells, UVA irradiation induced a significant decrease in the percentage of DDC that acquire co-stimulatory molecules (CD40, CD80, CD86) as well as maturation markers (CD83

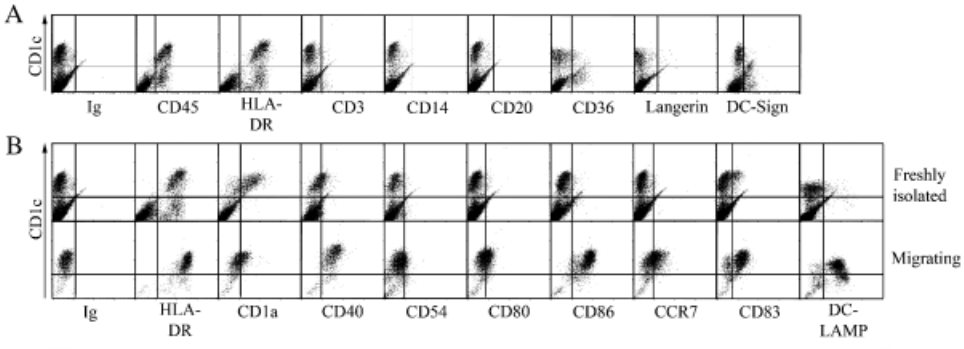

Figure 3
Phenotypic profiles of resident *versus* migrating human dermal dendrtic cell (DDC). (*A*) Human dermis was digested for 2 h with an enzymatic cocktail. The resident dermal cells were recovered and double stained with anti-CD1c and the indicated monoclonal antibodies (mAb). Cells were analyzed by flow cytometry, without any gating. (*B*) Activation/maturation status of freshly isolated *versus* migrating DDC. Dermal cells were recovered either after dermis digestion or after a 2-d migration from dermis. Cells were double stained with anti-CD1c and the indicated mAb and analyzed by flow cytometry.

Table I. Phenotype of migrating dermal dendritic cells

	DR	CD1a	CD40	CD54	CD80	CD86	CD83	CCR7	DC-LAMP
n	4	4	6	5	5	6	6	5	4
Non-irradiated	97.8 ± 1.4	69.2 ± 15.5	67.5 ± 18.5	41.4 ± 10.1	46.8 ± 12.3	89.7 ± 4.5	68.8 ± 8.5	55.7 ± 16	67.6 ± 8.1
2J/cm^2	94 ± 3.8^a	62 ± 17.6^a	56.7 ± 23.1^b	36.4 ± 14.4	30.8 ± 12^c	79.3 ± 12.2	56.8 ± 10.6^a	45.7 ± 18.2	55.9 ± 6.8
12J/cm^2	86.1 ± 15.4	48.6 ± 21.3^a	44.2 ± 23.8^b	37 ± 18.4	20.4 ± 13.7^b	68.2 ± 15.3^b	38 ± 12.9^c	37 ± 15.9^a	53.6 ± 10.9

Human dermis was irradiated, or not, with UVA at 2 or 12 J per cm^2 and incubated for 48 h in serum-free medium. Migrating cells were then recovered and CD1c$^+$ population was gated and analyzed for activation/maturation markers. Data represent the mean percentage of positive cells (± SD) and n gives the number of independent experiments. Statistical significance was assessed using the Student's *t* test. Values of $p<0.05$ were considered statistically significant, $^a p<0.05$, $^b p<0.01$, $^c p<0.005$.
UVA, ultraviolet A.

and CCR7). These modifications were concentration dependent and already observed after the lower dose of irradiation for CD40, CD80, and CD83 antigens. Similarly, the percentage of HLA-DR and DC-LAMP CD1c$^+$ cells tended to decrease on irradiated DDC. The percentage of CD1a$^+$ DDC only decreased after dermis irradiation at 12 J per cm^2. The mean fluorescence intensity of all antigens was consistently, although not significantly, decreased, suggesting that some DDC could escape the UVA deleterious effect (not shown). It is of note that gating out the dead cells using propidium iodide did not significantly alter the results, suggesting that the decreased number of mature DDC after irradiation did not merely reflect UVA-induced necrosis (not shown).

In order to assess whether UVA-induced altered DDC maturation could be related to increased apoptosis, double staining was carried out using FITC–annexin. Upon UVA irradiation, most CD1c$^+$/annexin$^+$ apoptotic cells failed to acquire the activation/maturation markers CD83 or CD86 (not shown).

UVA alters the allostimulatory capacity of human DDC Emigrant dermal cells from irradiated or sham-irradiated dermis were then evaluated for their capacity to induce allogeneic T cell proliferation. To this end, graded numbers of viable dermal cells were added to T cells. As shown in Fig 4*A*, the total dermal cell allostimulatory function was significantly reduced after irradiation at 12 J per cm^2. The results were still more significant when corrected according to the number of viable DDC, i.e. CD1c$^+$/IP- cells, in the suspensions (Fig 4*B*). The results demonstrated that UVA irradiation decreased the ability of DDC to present antigen to T lymphocytes and therefore reduced the efficiency of immune response.

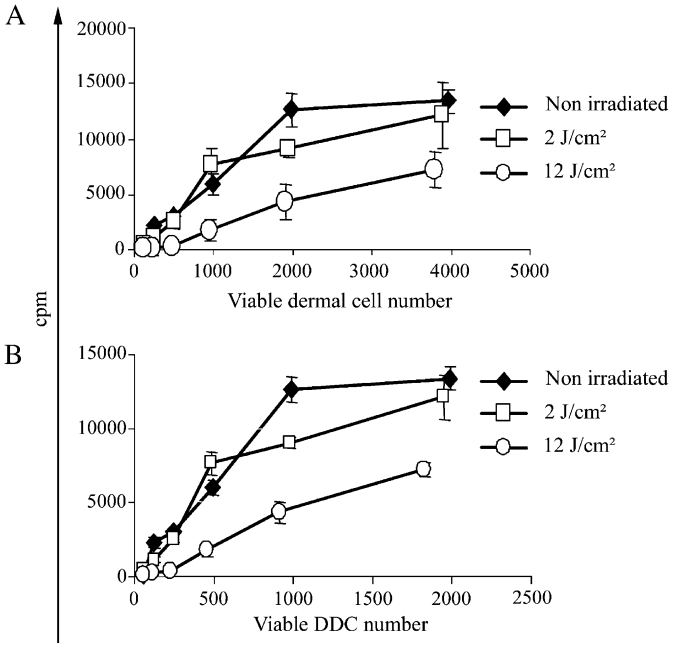

Figure 4
Ultraviolet A (UVA) alters the allostimulatory function of human dermal dendrtic cell. Human dermis was irradiated, or not, with UVA at 2 or 12 J per cm^2 and incubated for 48 h in serum-free medium. Migrating cells were then recovered, and graded number of viable cells was added to purified allogeneic T cells. After 5 d, T cell proliferation was assessed by the addition of 3H-thymidine for 18 h. Results are the mean ± SD of triplicate wells and representative of four experiments. The cpm of T cells alone never exceeded 50. The stimulator cell numbers correspond to either total migratory dermal cells (*A*) or was corrected according to the percentage of viable CD1c$^+$ cells in the suspensions, as assessed by flow cytometry analysis (*B*).

Discussion

The aim of this study was double: (1) to extend the characterization of human dermal DC and (2) to analyze the effects of UVA irradiation on the phenotypic and functional properties of these cells. To this end, dermal sheets were irradiated and DC collected by using their spontaneous migratory property.

The technique we used to recover emigrant dermal cells was similar to those previously described (Lenz *et al*, 1993; Nestle *et al*, 1993), except that we used serum-free medium and left the dermal pieces whole instead of cutting them into small fragments. This, respectively, avoids serum factors that might induce DDC maturation and substantially decreased the relative number of non-hematopoietic cells in the migratory cell suspension (personal observation, not shown).

Dermal resident or emigrant DC had been most frequently identified using HLA-DR and/or CD1a expression (Lenz *et al*, 1993; Meunier *et al*, 1993; Nestle *et al*, 1993; Sepulveda-Merrill *et al*, 1994; Turville *et al*, 2002). Apart from DDC, however, HLA-DR stained many migratory T cells and monocytes, as well as resident non-hematopoietic dermal cells. Moreover, as opposed to CD1c, CD1a expression decreased upon DDC migration. This might explain why a subset of CD1c$^+$ cells was CD1a$^-$. We found here that anti-

CD1c mAb most selectively identify DDC and might therefore be very useful for the cell purification. The results agree with *in situ* staining that reveals far higher reactivity in the dermis using anti-CD1c, as compared with anti-CD1a mAb (Nestle *et al*, 1993; Gerlini *et al*, 2001). Moreover, previous studies showed that anti-CD1c mAb react with all emigrant dermal DC cells, purified by cell sorting according to high forward and side scatter properties (Meunier *et al*, 1993), or selected on HLA-DR^{bright} expression (Gerlini *et al*, 2001). Lastly, the allostimulating property of dermal DC has been related to the CD1c$^+$ subset (Meunier *et al*, 1993).

Mainly because of CD1 expression, the bulk of dermal DC has long been related to epidermal LC. Indeed, it was thought to be LC precursors on their way to the epidermis and, especially, epidermal LC on their way to lymph nodes. The recent discovery of Langerin (Valladeau *et al*, 2000), which selectively identifies LC, revealed that only scarce Langerin-expressing cells were present in the dermis, as assessed by immunohistological studies of both murine and human skin (Valladeau *et al*, 1999; Ebner *et al*, 2004). In agreement with a recent paper (Ebner *et al*, 2004), we confirmed here, by flow cytometry, that very few migrating DDC were Langerin$^+$ and extended the results to resident DDC. Since the antigen was substantially expressed on LC after extraction and *in vitro* culture (Stoitzner *et al*, 2003; and personal observation) it is unlikely that the antigen may be lost on dermal DC following *in vitro* preparation or migration from the dermis. Finally, the recently described very low turnover of LC cells under steady state (Merad *et al*, 2002) is in line with the assumption that most dermal DC represent a resident population of DC.

In a recent paper, Larregina *et al* (2001) described a CD14$^+$/Langerin$^+$ population that migrated from whole-skin explants and differentiated into LC upon *in vitro* culture. Here we found some migrating CD14$^+$ cells but, in agreement with others (Ebner *et al*, 2004), we never detected Langerin expression in these cells.

CD36$^+$ cells were known to re-colonize the epidermis following UVB-induced LC depletion (Cooper *et al*, 1986). As opposed to Lenz *et al* (1993), who found that all DDC expressed CD36 by gating the cells according to high HLA-DR expression, we here reported CD36 expression on a sub-population of CD1c$^+$ or CD1a$^+$ DDC, suggesting that these cells might be LC precursors.

Only few data are available concerning the activation/maturation status of human DDC. Our observations confirm and extend previous studies showing that the bulk of resident DDC are immature. In contrast, most migrating DDC strongly expressed the maturation marker DC-LAMP as well as CCR7, a prerequisite for skin DC migration to occur (Ohl *et al*, 2004). This supports a full role of dermal DC population in skin immune responses. Inasmuch as these cells differ from the epidermal DC, especially regarding C-type lectins as well as CD1 expression (Pena-Cruz *et al*, 2003; Ebner *et al*, 2004), the respective role of each cell population in the skin immune response remains to be defined. For example, a recent paper demonstrated that, in mice, delivery of a protein antigen into the dermis gave predominantly Th1 responses, whereas antigen presentation by LC was strongly Th2 biased (Strid *et al*, 2004).

As opposed to UVB, a large part of solar UVA radiation enters the human dermis. There is now strong evidence that UVA contributes to the development of skin cancers (Kelfkens *et al*, 1991; Wang *et al*, 2001), but its effect on the immune system is less well defined. Despite conflicting data in the literature, showing that a high dose of UVA can protect hairless mice from UVB-induced immune suppression (Reeve *et al*, 1998), there has recently been a greater awareness regarding its immunosuppressive role. For example, UVA was shown to augment solar-simulated UV-induced local suppression of recall responses to nickel in humans (Kuchel *et al*, 2002). The mechanism was thought to involve LC, the epidermal DC known to play a crucial role in the initiation of skin immune responses. Indeed, UVA irradiation decreased the number of LC in human epidermis (Dumay *et al*, 2001) and was shown to inhibit the *in vitro* allostimulatory (Clement-Lacroix *et al*, 1996) or hapten-presenting function of LC (Iwai *et al*, 1999) in a concentration-dependent manner, a process accompanied by suppression of CD54, CD80, and CD86 co-stimulatory molecules. *In vivo*, UVA-II (320-340 nm) exposure reduced the skin's ability to mount contact sensitization and induced active suppression, a process related to impaired LC antigen-presenting function (LeVee *et al*, 1997).

We showed here that UVA irradiation of human dermis impaired the phenotypic and functional maturation of migrating dermal DC into potent antigen-presenting cells and induced their apoptosis. This might suggest that, under *in vivo* conditions, few efficient DDC would be able to reach the lymph nodes. The dose of UVA that we used can be considered as physiologically relevant since about 15 J per cm^2 UVA were received after about fifty minutes exposure to sunlight. We found that, upon UVA irradiation, the number of viable emigrant dermal DC was reduced, corresponding to a cytotoxic effect of UVA, as assessed by annexin staining. As a consequence, a significant proportion of the viable DC migrating after UVA irradiation failed to acquire the activation/maturation markers such as CD40, CD86, CD83, CCR7, and DC-LAMP. This ultimately correlates with an impaired capacity of the cells to induce allostimulatory T cell response.

Since this experimental approach quite eliminates the epidermal compartment, one could question its physiological relevance. Accordingly, some experiments have been carried out using whole-skin samples and triple staining. Skin was irradiated at 15 and 18 J per cm^2, a lower dose than that applied to dermal sheets, considering that about 40% of UVA radiation reach the dermis. The FSC/SSC profile of whole-skin-migrating cells was very similar to that obtained with dermal sheets, therefore allowing the elimination of T and B cells for subsequent analysis. By gating DDC according to CD45$^+$/Lang$^-$ staining, we found that skin irradiation at 15 and 18 J per cm^2 induced a decrease in the percentage of CD1a, CD80, CD83, CD86, and CCR7 expressing DDC, very similar to that obtained after dermal sheet irradiation. The results suggest that the presence of the epidermal compartment did not prevent the deleterious effects of UVA on DDC, and might even strengthen its effect.

Thus, under UVA exposure, alterations of both epidermal and dermal DC would lead to an alteration of immune response. With regard to immune suppression, UVA might be therefore more harmful than UVB that would only alter ep-

idermal LC. In line with this, recent studies showed that UVA protection predicts the ability of sunscreens to provide immune protection in humans (Damian *et al*, 1997; Moyal and Fourtanier, 2001; Nghiem *et al*, 2001; Baron *et al*, 2003; Poon *et al*, 2003). Moreover, when UVB were removed from solar-simulated UV radiation, there was no observable effect on the suppression of delayed-type hypersensitivity in mice, therefore demonstrating that UVA effects dominate those of UVB (Nghiem *et al*, 2001; Halliday *et al*, 2004).

Many of the biological effects of UVA are caused by reactive oxygen species (Halliday *et al*, 1998). Indeed, exogenous glutathion, as well as vitamin E, were found to reverse the UVA-induced suppression of LC function (Clement-Lacroix *et al*, 1996). Although the mechanisms involved in dermal DC alteration need to be clarified, this suggests that UVA may alter the immune system via an oxidative pathway.

In conclusion, the results showed that UVA impairs the phenotypic and functional maturation of human DDC, a full DC population that represents a second line of defence behind epidermal LC. This may be, therefore, an additional mechanism by which solar radiation impairs immune response and subsequently induces skin cancer growth. This mechanism might be especially important during the aging process, characterized by thinner epidermis and thus increased UVA sensitivity and might explain, at least in part, the increased frequency of cutaneous cancers in the elderly. The results underline the importance of protecting the population not just from UVB but also from UVA irradiation.

Materials and Methods

Preparation of dermal sheets Normal skin specimens were provided from plastic abdominal surgery after obtaining the patients' informed consent. Human skin was freed of fatty tissue and split cut with a keratome set. The resulting skin stripes were incubated in Hank's Balanced Salt Solution (HBSS) (Gibco, Cergy Pontoise, France) containing 1% gentamycin (Sigma, L'isle d'Abeau Chesnes, France) and 0.25% dispase II (Roche, Meylan, France) during 1 h at 37°C in 5% CO_2. Epidermal sheets were then stripped off the dermal layer using forceps. Dermal pieces were washed twice in HBSS, 1% gentamycin. This protocol was approved by the institutional review board of the University Claude Bernard Lyon1 and is in accordance with the Declaration of Helsinki.

Irradiation of dermal sheets Dermal sheets were layered into 100 mm ∅ Petri dishes in *X-Vivo* medium (Cambrex, Emerainville, France), 1% gentamycin and exposed to different doses of UVA radiation. The UVA source was a bank of four tubes emitting from 320 to 400 nm with a peak at 365 nm (Biosun apparatus, Vilber Lourmat, Marne la Vallée, France). The detector is a radiometer with a precision of ± 5%, according to the reference radiometer VLX1.3W. The precision of the intensity measure is ± 0.2%. The mean distance from the lamp to the irradiated cells is 23 cm. Two doses were used in this study: 2 and 12 J per cm^2. After irradiation, or after a corresponding sham-irradiation period (control), each sample was incubated at 37°C in 5% CO_2 during 48 h for cell migration.

Collect of emigrant dermal cells Non-adherent cells that had migrated out of the dermal explants were collected by filtrating the culture medium through a sterile gauze. The explants were washed twice, and the emigrant cell suspension was obtained after a 10 min centrifugation at 400 × *g*. The viability of cell suspensions from UVA- or sham-irradiated dermal sheets was determined by trypan blue exclusion.

Preparation of dermal cell suspension In some experiments, cells were extracted from freshly prepared dermis. To this end, dermal sheets were incubated during 2 h at 37°C, under gentle agitation, in RPMI-1640 (Gibco), 1% gentamycin, 1 mg per mL collagenase (Sigma), 1 mg per mL hyaluronidase (Sigma), and 100 μg per mL DNaseI (Sigma). The digestion medium was then filtrated through a sterile gauze to obtain total dermal cell suspension.

Flow cytometry Cells were incubated for 30 min at 4°C with affinity-purified mAb at the appropriate concentration or with irrelevant isotype-matched mouse Igs at the same concentration. In some experiments, viable cells were analyzed excluding PI-positive cells. The following mAb were used: anti HLA-DR-FITC (B8.122), anti CD54-FITC (84H10), anti CD80-FITC (MAB104), anti CD83-FITC (HB15a), anti DC-LAMP-PE (104.G4), anti-Langerin-PE (DCGM4), all from Immunotech (Marseille; France), anti CD40-FITC (clone EA-5) from Biosource International (Nivelles, Belgium), anti CD45-FITC (HI30), anti CD86-FITC (FUN-1), anti CD19-FITC (HIB19), all from BD Biosciences (Pont de Claix, France), anti CCR7-FITC (150503), anti DC-SIGN-FITC (DCN46) from R&D system (Lille, France), anti CD1a-FITC (clone NA1/34), anti CD3-FITC (UCHT1), anti CD14-FITC (TÜK4), anti CD16-FITC (DJ130c), anti CD20-FITC (B-Ly1), all from DAKO (Trappes, France), and anti CD1c-PE (AD5-8E7) from Miltenyi Biotec (Paris, France).

For double-color fluorescence, FITC- and PE-conjugated mAb were incubated at the same time. Intracellular stainings were performed using the Fix and Perm kit from DAKO. After incubation with the antibodies, cells were washed and re-suspended in phosphate-buffered saline, 1% bovine serum albumin, and 0.02% sodium azide. Fluorescence analysis was performed on a FACScan using LYSIS II software (BD Biosciences).

Apoptosis Quantification of apoptotic cells was assessed using an apoptosis kit (Immunotech). The early exposure of phosphatidylserine residues at the surface of apoptotic cells was measured using Annexin V–FITC staining, with dead cells identified by PI.

Allogeneic T cell proliferation assays Human T cells were purified from normal human blood by the rosetting method, using 2-amino-ethylisithiouronium bromide hydroxy-bromide-treated sheep red blood cells, as previously described (Kaplan and Clark, 1974). Graded numbers of viable dermal cells were used as stimulators of 10^5 T cells in RPMI-1640 medium supplemented with 10% normal human AB serum (EFS, Lyon, France). Cultures were established in triplicate and maintained in 96-well round-bottomed plates at 37°C. After 4 d of culture, lymphocyte proliferation was measured by pulsing the cells with 1 μCi of [^{3}H] methylthymidine (5 Ci per mmol, Amersham, Les Ulis, France) for the final 18 h of culture. Cells were then harvested and incorporated thymidine was quantitated in a direct beta counter (Matrix 96, Packard Instruments, Meriden, Connecticut). Results were expressed as the mean counts per minute (cpm) + SD of triplicate cultures.

Statistical analysis Results were analyzed for statistical significance using Student's *t* test. Values of $p < 0.05$ were considered statistically significant.

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